

**WHAT IS CLAIMED IS:**

1. (currently amended) A method for creating a functionally active chimeric ~~altering the cleavage specificity of a~~ Type IIG restriction endonuclease, ~~the Type IIG restriction endonuclease characterized by a cleavage domain adjacent to a methylase domain, the methylase domain located adjacent to a specificity domain,~~ containing in order: a cleavage domain, a gamma type methylase domain, and a specificity domain, the method comprising:

(a) ligating a first DNA sequence and a second DNA sequence to form a ~~fusion~~ recombinant DNA, wherein

(i) the first DNA sequence comprises a DNA segment encoding a ~~catalytic~~ cleavage domain and an N-terminal portion of a methylase domain ~~of~~ for a first Type IIG restriction endonuclease, and

(ii) the second DNA sequence, comprises a DNA segment encoding a specificity domain and a C-terminal portion of a methylase domain of a second Type IIG restriction endonuclease;

such that the ligation occurs between sequences encoding the methylase domain of (i) and (ii) to form a fusion junction in the chimeric endonuclease encoded by the ligated DNA; and

(b) transforming a host cell with the ~~fusion~~ recombinant DNA ~~for expressing a~~ to express the functionally active chimeric Type IIG restriction endonuclease ~~with altered cleavage specificity.~~

2. (original) A method according to claim 1, wherein step (a) further comprises: introducing a mutation into the cleavage domain to enhance the viability of the transformed host cell.

3. (currently amended) A method according to claim 1, wherein the ~~sequence corresponding to the N-terminal portion of the methylase terminates in a methylase conserved motif selected from motifs X, I, II, III, IV, V, VI, VII or VIII.~~ fusion junction occurs proximate to or within (i) a conserved amino acid sequence in a methylase motif or (ii) a boundary between the methylase domain and the specificity domain; wherein the methylase motif is selected from the group consisting of motifs X, I, II, III, IV, V, VI, VII or VIII.

4-5. (cancelled)

6. (currently amended) A method according to ~~claims 3 or 5,~~ claims 1 or 3, wherein the fusion junction is located ~~sequence corresponding to the N-terminal portion of the methylase motif terminates~~ between the sequence encoding motif III and NPPY in motif IV.

7. (original) A method according to claim 1, wherein ligation occurs by means of a linker sequence attached to each of the N-terminal portion of the methylase domain and the C-terminal portion of the methylase domain on the first and second DNA segment.

8. (currently amended) A method according to claim 1, wherein the ~~fusion~~ recombinant DNA encodes an active methylase domain.

9. (original) A method according to claim 1, wherein the first and second Type IIG endonucleases have defined cleavage and recognition sites.

10. (currently amended) A method according to claim 1, wherein the first Type IIG endonuclease has a defined cleavage domain and

~~recognition-site~~ specificity domain and the second Type IIG endonuclease is characterized by a bioinformatic search of a microbial sequence database.

11. (currently amended) A method for obtaining a functionally active chimeric Type IIG restriction endonuclease, ~~forming a non-natural, functional Type IIG restriction endonuclease, wherein the Type IIG restriction endonuclease is characterized by a functional~~ containing a cleavage domain, a functional Gamma type methylase domain and an altered functional a specificity domain, compared with a natural form of the functional Type IIG endonuclease; the method comprising:  
(i) expressing in a host cell, a recombinant DNA encoding the chimeric restriction endonuclease, wherein the recombinant DNA is formed from a first DNA fragment encoding the cleavage domain and optionally a portion or all of the methylase domain of a first Type II G restriction endonuclease and a second DNA fragment encoding the specificity domain of a second Type IIG restriction endonuclease;  
wherein the first and second fragments of DNA are obtained by (i) selecting primers for amplifying the first and second DNA fragments by two-step PCR, to form the chimeric Type IIG restriction endonuclease;  
or (ii) cleaving the DNA encoding the first and second Type IIG restriction endonuclease with one or more restriction endonucleases and ligating the cleaved DNA to form the chimeric Type IIG restriction endonuclease.

~~(a) inserting into a DNA encoding the methylase domain or the specificity domain of the natural form of the functional Type IIG endonuclease, a mutation or a nucleic acid linker sequence for inactivating optionally the cleavage domain and inactivating (i) the functional methylase domain and the specificity domain or (ii) the functional methylase domain or the specificity domain;~~

~~(b) ligating to the DNA at the mutation or at the linker, a DNA encoding (i) a portion of the methylase and specificity domain or (ii) a portion of the methylase or specificity domain to form a fusion DNA; and~~

~~— (c) transforming a host cell having a marker for detecting a colony expressing a non-natural functional Type IIG restriction endonuclease.~~

12. (currently amended) A method according to claim 11, ~~wherein the mutation is positioned~~ further comprising: ligating the first and second DNA fragments at a site corresponding to within a conserved motif in the methylase domain.

13. (currently amended) A method according to claim 11, ~~wherein the mutation is a deletion at a~~ further comprising: ligating the first and second DNA fragments at a site proximate to or within a site corresponding to the N-terminal 5'- end of the DNA encoding the specificity domain.

14. (cancelled)

15. (currently amended) A method according to claim 11, wherein at least one of the first DNA fragment and the second DNA fragment has a linker. ~~wherein the linker is a transposon-mediated linker insertion sequence.~~

16. (currently amended) A method according to claim ~~11~~15, wherein the linker contains a restriction endonuclease cleavage site, the

Shuang-Yong, Xu et al.  
Serial No.: 10/800,946  
Date Filed: March 15, 2004  
Page 6

cleavage site being ~~which~~ is unique within the DNA encoding the restriction endonuclease.